Butler University Botanical Studies
Volume IV *Papers No. 6 and 7*
December, 1938

A Quantitative Study of the Phytoplankton of Lake Michigan Collected in the Vicinity of Evanston, Illinois

WILLIAM ALLEN DAILY

A Comparison of Market Milk from Ten Indianapolis Companies by Use of the Direct Microscopic Method of Analysis

INA STANLEY

These papers are contributions No. 92, 93, respectively, from the botany laboratories of Butler University. Address all communications regarding them to Butler University Botanical Studies, Butler University, Indianapolis, Indiana, U. S. A.

BUTLER UNIVERSITY BOTANICAL STUDIES

Butler University Botanical Studies are offered in exchange for publications of learned societies and institutions, universities and libraries, or they may be purchased at $2.00 per volume. Applications should be made to the Department of Botany, Butler University, Indianapolis, Indiana, U. S. A.

VOLUME I

1. Some ecological factors in secondary succession: Upland hardwood. I. Evaporation studies in the Sycamore Creek region, by Stanley A. Cain and Ray C. Friesner. Pp. 1-16. March, 1929.
2. Some ecological factors in secondary succession: Upland hardwood. II. Soil reaction and plant distribution in the Sycamore Creek region, by Stanley A. Cain and Ray C. Friesner. Pp. 17-28. May, 1929.
3. The genus Trillium in Indiana, by Ray C. Friesner. Pp. 29-40. May, 1929.
4. Life forms and biological spectra of the flora of Bacon's swamp, Indiana, by Alice Phillips. Pp. 41-54. July, 1929.
5. Key to genera of ferns and fern allies, by Ray C. Friesner. Pp. 55-60. July, 1929.
6. The relation of certain ecological factors to the inhibition of forest floor herbs under hemlock, by Rexford F. Daubenmire. Pp. 61-76. January, 1930.
7. Chromosome numbers in ten species of Quercus with some remarks on the contributions of cytology to taxonomy, by Ray C. Friesner. Pp. 77-104. January, 1930.
8. A study of fruit diseases occurring in a mid-western market, by George W. Fischer. Pp. 105-128. March, 1930.
9. Certain floristic affinities of the trees and shrubs of the Great Smoky mountains and vicinity, by Stanley A. Cain. Pp. 129-156. September, 1930.
10. A comparison of strip and quadrat analyses of the woody plants on a central Indiana river bluff, by Stanley A. Cain, Ray C. Friesner and John E. Potzger. Pp. 157-171. October, 1930.
11. Certain aspects of the H-ion concentration of the soils of a central Indiana river bluff, by Stanley A. Cain and Ray C. Friesner. Pp. 172-175. October, 1930.
12. A microtome knife cooler, by Ray C. Friesner. P. 176. October, 1930.
13. An ecological study of the heath balds of the Great Smoky mountains, by Stanley A. Cain. Pp. 177-208. December, 1930.

VOLUME II

1. Algæ of Marion county, Indiana, a description of thirty-two forms, by C. Mervin Palmer. Pp. 1-24. February, 1931.
2. The acid ranges of some spring-flower herbs with reference to variations in flower color, by Rexford F. Daubenmire. Pp. 25-28. August, 1931.
3. Factors favoring the persistence of a relic association of eastern hemlock in Indiana, by Rexford F. Daubenmire. Pp. 29-32. August, 1931.
4. Fat deposits in certain Ericaceæ, by Oran B. Stanley. Pp. 33-44. August, 1931.
5. Chromosome numbers in *Fagus grandifolia* and *Quercus virginiana*, by Hellen Aufderheide. Pp. 45-52. October, 1931.

Continued on Inside Back Cover

A QUANTITATIVE STUDY OF THE PHYTOPLANKTON OF LAKE MICHIGAN COLLECTED IN THE VICINITY OF EVANSTON, ILLINOIS[1]

By WILLIAM ALLEN DAILY

There have been but few papers published in which a quantitative study of the phytoplankton of Lake Michigan was considered. A study of this nature was therefore undertaken, using the Sedgwick-Rafter method.

A brief summary of the quantitative and qualitative studies which have been published on Lake Michigan includes the following: Briggs (1872) and Thomas and Chase (1887) presented lists of Diatomaceæ found in Lake Michigan. Later in 1896, reports were published by Ward, Thompson, and Kofoid which contained phytoplanktonts and some protozoans, as well. Other reports by Kofoid (1896) and Jennings (1896) dealt with the Rotatoria.

In 1927, Eddy published a list of both phytoplankton and zoöplankton of Lake Michigan and their relative abundance and seasonal variation. He writes: "There appeared to be a fairly constant and uniform phytoplankton throughout the year, although the zoöplankton showed some response to seasonal conditions. Diatoms predominate at all times and constitute the majority of the organisms of the plankton, the same species being conspicuous in all collections examined." His conclusions were based upon two series of collections made from Lake Michigan in 1887-1888 and 1926-1927, of which the former were made by the silk-net and filter-paper method. All of these were surface tows near the shore and were collected at Chicago, Illinois; Sawyer, Michigan; Michigan City, Indiana; and Indiana Dunes State Park.

Baylis and Gerstein (1929) list both zoöplanktonts and phytoplanktonts found in the lake water of the Chicago water supply. They found that in a two-year study of Lake Michigan the peak of plankton periodicity was in May 1927 and again in September 1928. In 1927 a second but lesser peak came in October. Temperature and sunlight readings were included, but no attempt was made to correlate these

[1]A portion of the work done on a thesis in partial fulfillment of the requirements for the Master of Science Degree in Northwestern University. The pages of Butler University Botanical Studies are open to all Butler University alumni.

with the periodicity of the plankton. Samples were collected five times a week at first, but later only three times.

Ahlstrom (1936) published a detailed report on the deep water and inshore plankton of Lake Michigan at Evanston; Crustacea were excluded. All collections were made by the silk-net method. Burkholder (1929) found that there is an autumn maximum of diatoms in Lake Erie. Gottschall and Jennings (1933) found that diatoms were the most abundant group of phytoplankton in Lake Erie and that they occurred in two peaks of abundance, one in the spring and one in the fall. They did not find the Chlorophyceæ and the Myxophyceæ abundant. Tiffany (1938) reports a definite autumnal maximum of Myxophyceæ in the west end of Lake Erie.

West and West (1913), in a study of English lakes, report that the greatest amount of phytoplankton is found in late summer and autumn, during the autumnal decline in temperature.

Inasmuch as phytoplankton is affected by various ecological factors, several of these have been considered in this paper, *e. g.*, temperature, turbidity, hydrogen-ion concentration, bacteria, and sunlight. It is generally assumed by most writers that temperature plays the leading role in algal and especially diatom periodicity, provided other factors do not limit the growth of the plants. Allen (1920) published an exhaustive analysis of the plankton of the San Joaquin River in California. He found the diatoms dominating the phytoplankton and concluded that temperature is the principal factor determining the plankton periodicity. Also he points out that the river shows a plankton maximum in the autumn. Eddy (1930) states that the rate of fresh-water plankton reproduction and consequently the abundance at different seasons in the same body of water varies directly with the temperature. Roach (1932) reports in his study on river plankton, that the phytoplankton varies in direct ratio with the temperature. Coffing (1937) states that temperature seems to be a primary factor influencing production in canal phytoplankton.

Pearsall (1923), on the other hand, does not believe that temperature plays the leading role in diatom periodicity, but that deficiencies of oxygen, nitrates, silica, or calcium are usually the limiting factors. He points out that floods influence the amount of these dissolved substances in the water. Later, Pearsall (1932) writes that diatoms occur in winter and spring when nitrates, phosphates, and silica are in abundance, and that the green algæ occur in the summer when nitrates and phosphates are low.

The subject of turbidity in relation to seasonal abundance of phytoplankton has received little attention from limnologists and algologists. Eddy (1930), however, regards turbidity as an important factor in reducing light and hindering the development and movements of many planktonts. He suggests that conditions must be such as to reduce this turbidity to proper value before plankton production can be heavy.

Hydrogen-ion concentration has received much attention in the past, but now is considered more of an index of a general environmental condition than a controlling factor in determining the periodicity of phytoplankton. Gottschall and Jennings (1933), in their study of Lake Erie, found that pH varied with the free carbon dioxide content. The lowest recorded pH was 7.6 and the highest 8.4. The pH value of the water studied by Eddy (1930) generally ranged from 7.8 in summer to 6.6 in winter, but the fluctuations were not always seasonal.

Although a number of papers have dealt with lake bacteria, few of these have considered their seasonal abundance in relation to that of phytoplankton. Recently, Henrici (1938) has reported on a periodical distribution of bacteria in relation to the periodical distribution of plankton. It was found that the numbers of bacteria followed closely the curve for total plankton, with a distinct lag. He concludes that the production of organic matter by plankton organisms is an important factor in determining the number of bacteria in the water.

According to Welch (1935), other workers studying the annual distribution of bacteria have found either one maximum and one minimum which do not necessarily occur at the same time in different years, or two maxima and two minima, the maxima occurring in the spring and autumn and the minima during the two stagnation periods.

METHODS

A weekly quantitative study of the phytoplankton of Lake Michigan at Evanston, Illinois, was made over a period of one year, May 1937 to May 1938. Several ecological factors were studied in conjunction with the seasonal periodicity of the phytoplankton, *e. g.*, temperature, turbidity, hydrogen-ion concentration, bacteria, and sunlight.

Essentially, the Sedgwick-Rafter procedure was used in this study: (1) collection of lake water samples; (2) filtration and concentration; (3) examination and enumeration; (4) calculation of the number of phytoplankton organisms per ml.

Collection

Collection of two duplicate samples each week was made over a period of one year beginning May 9, 1937, and terminating May 3, 1938.[1] These collections were taken from the end of a breakwater which extends 65 meters into the lake, and which is situated on the Northwestern University campus. The water is 2 meters deep at the point of collection, but this fluctuates with the weather conditions. One one-half gallon Mason jar was fastened in an improvised "holder" at the end of a pole, and was then plunged beneath the surface of the water, not exceeding 3 decimeters. The collected water was poured into a second jar and the first was refilled. In several instances, openings were made in the ice to obtain samples, and once, because of inclement weather, a sample was taken only a few yards from the shore.

Filtration and Concentration

Samples were brought immediately into the laboratory and 1000 ml from each jar were poured into separate graduated cylinders. In several cases, only 500 ml and once 700 ml were filtered because of high turbidity. These were then emptied slowly into two filter funnels of 500 ml capacity. Each of these was fitted with a one-hole rubber stopper with a U-tube inserted. 13-19 mm of 60-120-mesh filter sand were separated from the tube and stopper by a 200-mesh silk bolting cloth disc about 12 mm in diameter. A filter pump was used at all times to diminish the time of filtration, which was usually about three hours. This was deemed necessary because of the high turbidity which occurred in most of the samples. The inner surface of the funnels was washed down occasionally with distilled water to remove organisms and debris. The surface of the sand was intermittently broken by use of a fine needle fastened in the end of a glass tube. This was done in order to hasten filtration.

Following filtration, the sand and residue were washed directly into a small bottle by means of 10 ml of Transeau's preservative.[2] The bottles were immediately corked and sealed with paraffin for future study.

Examination and Enumeration

The usual procedure outlined by Whipple (26) was followed in the examination and enumeration of the phytoplankton. The Whipple ocular-micrometer was calibrated so that, with a 16 mm objective and a 10X

[1]Collections were not made the weeks of May 23-29, 1937, and December 26-January 1, 1938.
[2]Six parts water, 3 parts 95 percent ethyl alcohol, 1 part formalin.

ocular, any observed field of the counting chamber was exactly 1 sq mm. The counting chamber was 1 ml in capacity. In the enumeration, ten fields were counted in each sample. The ten fields were taken at random, attempting to distribute them equidistantly over the slide.

In counting the organisms, each planktont, regardless of the number of component cells, was counted as a unit. Rhizosolenia, Scenedesmus, Dinobryon, filaments of Fragilaria, Tabellaria, and Melosira were respectively counted as units. Likewise, a single cell of a colony or filament was counted as a unit if found separated from the original aggregate. As suggested by Todd and Sanford (*22*) for blood corpuscle enumeration, cells which touched the lower and right sides of each square were counted as if within the squares.

Genera and species are listed in those forms which can be definitely recognized with a 16 mm objective; however, it was impossible to identify correctly some genera and species with such magnification. Species were listed only when identification was comparatively certain. It was made a practice to examine, previous to the counting process, the duplicate concentrated sample and a net collection which was made the same day as the concentrated sample. This permitted checking of any unidentified species which might appear.

Calculation

The following formula was used in calculating the number of organisms per ml (Standard Methods of Water Analysis, 1936):

$$\frac{\text{No. of fields in a 1 ml counting cell 1 mm deep}}{\text{No. of fields counted}} \times \frac{\text{ml of concentrate}}{\text{ml of water filtered}} = \text{the multiplier}$$

thus:

$$\frac{1000}{10} \times \frac{10}{1000} = 1$$

The total number of organisms found in 10 fields in this case then equals the number of organisms in 1 ml of unconcentrated lake water.

Temperature

Temperature of the sample was taken immediately upon entering the laboratory with a minimal error, since the laboratory is only 175 feet from the lake shore, and since the sample bottle used for the reading was

allowed to remain submerged in the lake for a few minutes to assume the water temperature. Data for the period of May 1 to September 22, 1937, were secured from the Filtration Plant.

Other Factors

Turbidity was determined by use of standards prepared by the Evanston Filtration Plant according to Government Regulations. The standards ranged from 2 parts per million of fuller's earth to 50 parts per million and were prepared in glass-stoppered bottles. There were numerous times when the turbidity exceeded 50 and these were recorded as 50 plus. All standards were thoroughly shaken before comparing with the lake water of the same volume and contained in the same kind of bottle. Data for the period of May 1 to September 13, 1937, were secured from the Filtration Plant, whose standards were prepared in the same manner as those used in this study.

Hydrogen-ion concentration was determined with a LaMotte colorimetric outfit. Readings are recorded from the Filtration Plant from the period May 1 to September 6, 1937. Their determinations were made by using glass colorimetric standards.

Since the Filtration Plant also ran bacterial counts, an attempt has been made to see what correlation, if any, could be made between plankton and bacterial seasonal growth. The plate method was used for determining the number of bacteria in the water. Each plate contained 1 ml of lake water in 10 ml of nutrient.[1] Incubation was for 24 hours at 37° C. Each colony found was counted as one and considered as developing from one bacterium.

The total number of hours of sunshine per month was obtained from the United States Weather Bureau, Chicago, Illinois, through the kindness of the Water Purification Division of the City of Chicago.

THE EVANSTON WATER FILTRATION PLANT

The Evanston Water Filtration Plant is situated about three-fourths miles north from the station where the collections for this study were made. The information on raw lake water is computed from samples which are taken directly from the intake pipe line, which extends 1,680 meters into the lake. It is 30 inches in diameter and covered with a wir

[1]Nutrient was composed of agar, peptone, and beef extract.

grill having openings in it 2 inches square. An average of ten million gallons of water flow through the pipe every 24 hours.

Immediately the question arises as to the advisability of using in this paper data which were recorded at the above plant. The correlation between the records of the Filtration Plant and those of the author are given in table I.

TABLE I

Comparison of Data of Present Study with Data from Filtration Plant

	pH		Temperature		Turbidity	
	Filtration Plant	Present Study	Filtration Plant	Present Study	Filtration Plant	Present Study
October	7.6	7.8	15	17.3	8	
	7.6	7.8	13	11.5	30	
	7.6	7.8	11	10	8	15
	7.6	7.8	9	8.4	4	15
November	7.4	7.8	9	6.6	6	20
	7.8	7.8	8	8.2	6	10
	8.0	7.8	5.5	3.4	6	15
	7.6	7.8	4.5	1.5	6	30
December	7.4	7.8	.5	.1	8	15
	7.6	7.8	0.	0.	10	10
	7.4	7.6	0.	0.	6	15
	7.8					
January	7.6	7.6	.5	.1	6	6
	7.6	7.6	.5	.2	2	6
	7.6	7.6	1.	.2	4	15
	7.6	7.8	.5	0.	15	50+
February	7.6	7.6	.5	.5	8	15
	7.6	7.8	2.0	1.8	20	50+
	7.6	7.8	1.5	.9	20	30
	7.8	7.6	1.	1.1	20	50+

RESULTS AND OBSERVATIONS

In the 49 weekly samples taken from Lake Michigan, 43 genera representing 5 classes were recorded. Of these, 20 forms were determined to species. The classes were in order of numerical abundance, Bacillariophyceæ, Chrysophyceæ, Myxophyceæ, Chlorophyceæ, and Dinophyceæ. There was a maximum of 3,688 organisms per cc the week of June 4, 1937, and a minimum of 152 per cc the week of March 9, 1938. The maximal monthly average production of total phytoplankton occurred in June, and the minimal production in March.

The total phytoplankton (graph 5) showed two peaks of abundance, the first and greater in June 1937 and the second and lesser in October 1937, besides considerable weekly variation in number. These peaks correspond quite favorably with those found by Baylis and Gerstein (3) on Lake Michigan. The diatoms dominated the phytoplankton at all times, both in number and species. This is very similar to what Gottschall and Jennings (9) found in Lake Erie. The curve for diatoms (graph 6) conforms almost exactly with that of total phytoplankton.

The maxima in June and October are attributable mainly to Synedra, which was the most abundant diatom of the Bacillariophyceæ (table III) and was greatest numerically of all phytoplanktonts in June and again in October. Eddy's statement (8) that "seasonal variation in constituent species were noticeably lacking, the dominant diatoms running almost uniformly through the collection——," is not borne out by this paper. It is noteworthy, however, that these maxima were augmented by pulses of the dominant diatoms which were Asterionella, Fragilaria, Melosira, Synedra, and Tabellaria. Three other diatoms which were present throughout the year and nearly as abundant as the dominant group might be classed as "codominants." They are Cyclotella, Navicula, and Rhizosolenia.

Bacillariophyceæ

Each of the phytoplanktonts displayed its own pulse throughout the year. This was especially noticeable among genera in which more than one species were studied (table II). Among the dominant genera and species of diatoms at least one distinct pulse and sometimes more than one was found each month from June until December. The order Pennales (see classification list) led the Centrales in number and species at all times.

During the spring of 1937 the peaks of abundance of the dominant genera of diatoms occurred in the following order: Melosira in May, Asterionella and Synedra in June, and Fragilaria and Tabellaria in July. Gottschall and Jennings (9) found a somewhat similar succession in Lake Erie, with the exception that they found Tabellaria at the end of May.

Fragilaria and Tabellaria were both dominant diatoms, following Synedra in numerical abundance, and exhibited summer and autumnal seasonal maxima. The species of Fragilaria and Tabellaria showed uniformity to a rather high degree in their periods of abundance, but varied numerically (table II). It is noteworthy and demonstrated by Fragilaria that species of a genus are usually much unlike in number at the same

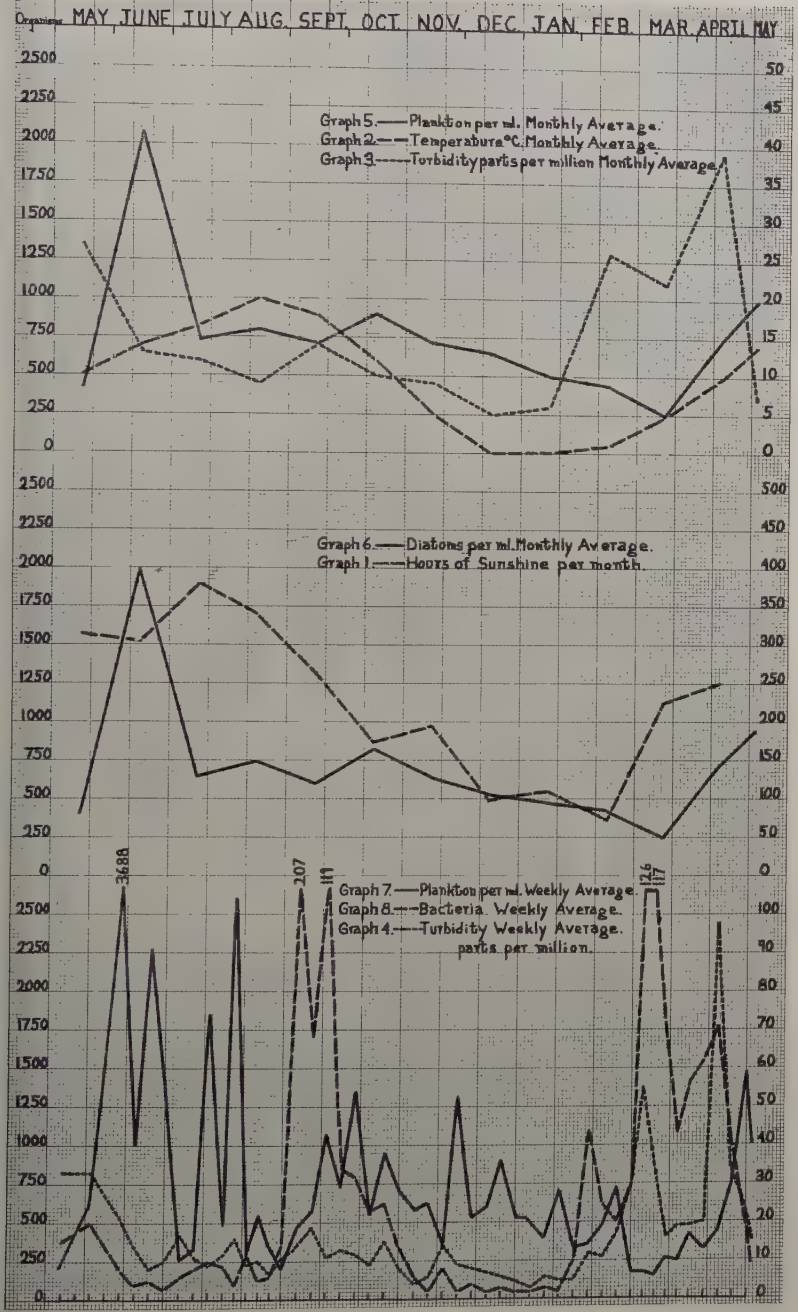
Organisms per ml.
MAY JUNE JULY AUG. SEPT. OCT. NOV. DEC. JAN. FEB. MAR. APRIL MAY
Graph 5.—Plankton per ml. Monthly Average.
Graph 2.—Temperature °C. Monthly Average.
Graph 3.—Turbidity parts per million Monthly Average.
Graph 6.—Diatoms per ml. Monthly Average.
Graph 1.—Hours of Sunshine per month.
Graph 7.—Plankton per ml. Weekly Average.
Graph 8.—Bacteria. Weekly Average.
Graph 4.—Turbidity Weekly Average.
parts per million.
3688
207
111
126
117

TABLE II

Diatom Genera and Species — Monthly Average

	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.	Jan.	Feb.	Mar.	Apr.	May
Asterionella	24	87	29	9	9	46	91	134	49	17	50	250	117
Fragilaria crotonensis	5	35	80	57	46	143	197	105	35	16	17	30	21
Fragilaria sp.	1	24	79	37	23	50	64	57	23	17	18	34	12
Fragilaria—Total	6	59	159	94	69	193	261	162	58	33	35	64	33
Melosira	77	58	18	12	10	20	5	5	5	10	51	208	173
Synedra ulna	6	21	4	3	4	10	9	6	5	17	10	12	0
Synedra sp.	261	1627	269	459	383	423	146	98	285	315	45	74	257
Synedra—Total	267	1648	273	462	387	433	155	104	290	332	55	86	257
Tabellaria flocculosa	4	9	51	21	8	18	15	6	5	2	2	6	11
Tabellaria fenestrata	8	20	64	48	23	38	27	24	8	6	8	16	24
Tabellaria—Total	12	29	115	69	31	56	42	30	13	8	10	22	35
Cyclotella	8	21	21	24	35	27	23	35	19	8	24	33	19
Rhizosolenia	1	25	23	13	6	12	32	25	17	11	9	51	294

weekly interval throughout the year. Tabellaria never dominated the diatoms numerically in any collection. Melosira was most abundant in the spring and fall months of 1937, and Asterionella was greatest in the spring and winter months, being dominant in only 3 weekly collections. Rhizosolenia was rather evenly distributed seasonally; however, there was a distinct spring maximum in 1938 and a slight winter increase (table II).

Cyclotella and Navicula, both codominants, exhibited similar spring and early fall periods of greatest abundance, which occurred in June and September respectively. The remainder of the diatoms were found in sparse numbers and occurred sporadically.

Chrysophyceæ

Three genera of the Chrysophyceæ (table III) were observed during the study, and all belonged to the same order, Chrysomonodales. One specimen each of Mallomonas and Chrysosphaerella were recorded. Dinobryon was found in all months except May 1937 and April 1938. It was at its peak in July, and this was followed by another lesser pulse in November. Baylis and Gerstein (3) report, "Dinobryon, and perhaps most animal organisms, usually have their peaks in the summer or fall."

Myxophyceæ

The Myxophyceæ appeared to have two periods of abundance, the higher in September and the lower in June. This appears to follow the

TABLE III

Phytoplankton Classes — Monthly Average

	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.	Jan.	Feb.	Mar.	Apr.	May
Bacillariophyceæ	403	2000	653	738	607	829	639	528	472	431	243	731	936
Myxophyceæ	14	57	17	22	83	31	26	6	2	2	2	12	48
Chlorophyceæ	1	8	2	2	4	6	4	5	5	0	4	8	15
Chrysophyceæ	0	19	66	38	20	20	62	41	15	1	1	0	0
Dinophyceæ	0	0	0	3	1	1	0	0	0	0	0	0	0

autumnal maxima of lake blue-greens, reported by the Wests (25), Whipple (26), and Gottschall and Jennings (9). Tiffany (19) reports a definite autumnal maximum of Myxophyceæ in the west end of Lake Erie. The order Hormogonales, represented by Anabæna, Lyngbya, and Oscillatoria in this study, was the outstanding group of the class, with Lyngbya being the dominant form. The Chroöcoccales, represented mainly by Cœlosphærium and Microcystis, were frequently present, but in few numbers. It is interesting to note (table III) that the blue-greens were almost as abundant numerically as the Chrysophyceæ, of which Dinobryon is outstanding.

Chlorophyceæ

Chlorophyceæ, represented mainly by Ankistrodesmus and Scenedesmus of the order Chlorococcales, was never abundant in the samples. The periods of amplitude occurred in June, October, and April; however, when the figures are low and comparatively alike, reasonable conclusions concerning seasonal growth cannot be made with certainty.

Dinophyceæ

Ceratium and Peridinium represented the class Dinophyceæ. Ceratium was present, 2 organisms per cc during August and one organism during September and October. Peridinium appeared only in August, one specimen having been observed. Gottschall and Jennings (9) only report Ceratium and Peridinium for Lake Erie and never very abundant.

Ecological Factors

It is thought that no one factor alone could be responsible for seasonal periodicity of plankton, and that a great number of known and perhaps unknown factors are necessary. It is important, however, in bodies of water such as Lake Michigan, to study each factor as far as possible

and determine what influence that factor may have on the periodicity of one or all organisms.

Obviously, the two "turnovers" of the lake have much to do with the spring and autumnal maxima of phytoplankton, by increasing the turbidity and thereby increasing the necessary gases and mineral salts, mainly oxygen, carbon dioxide, silicates, calcium, nitrates, and phosphates which are essential for optimum growth. Turbidity, monthly average, is shown in graph 3. Turbidity displayed two maxima, which were caused by the annual spring and fall lake turnovers. There were weekly fluctuations, however, which are mainly attributable to the weather conditions, such as storms and wind. Slight waves will cause an increase in turbidity near the shore. It appears from this study that the increase of turbidity during the month likewise influences phytoplankton pulses. From weekly studies (graphs 4 and 7), it was difficult to ascertain the exact relation between turbidity and plankton pulses; however, the increases in turbidity appear to precede the plankton pulses. The information here tends to lean toward Pearsall's (15) idea that temperature does not play a leading role in diatom periodicity, but, instead, the view that deficiency of oxygen, nitrates, silica, or calcium is usually the factor limiting diatom periodicity. Although temperature is important in optimum growth of most phytoplanktonts, it does not seem to be of prime importance as a controlling factor in diatom periodicity.

The highest temperature recorded was 21° C., which was reached August 1, 1937, and the lowest was 0° C., which occurred several times in December and once in February. Phytoplankton abundance and growth in the earliest spring seems to follow the rise in temperature rather closely (graphs 2 and 5). However, neither of the major plankton peaks occur near the temperature high. The average temperature in June, when the diatoms were at the period of greatest amplitude, was 7° C. lower than the highest temperature recorded. When the peak in October occurred, the temperature was 11.8° C. and was steadily declining at the time.

It is noticeable that the June and October phytoplankton maxima occur at approximately the same average temperature. However, it should be noted that the major weekly peaks occurred in a range from 1.5 to 21° C, so that no particular degree of temperature within this range may be designated as an optimum.

No correlation could be found between hydrogen-ion concentration and the seasonal periodicity of the phytoplankton. The pH varied from

pH 7.4 to 8.0 throughout the year, pH 8.0 occurring ten days in November 1937, and again, once only, on April 27, 1938. The rapid increase in phytoplankton at this time, and the few weeks preceding, might account for the reading of pH 8.0 on the basis of CO_2 utilization in photosynthesis. The author's pH recordings do not show a reading below 7.6.

The greatest number of hours of sunlight (graph 1) occurred in July 1937, and the least number in February 1938. The hours of sunlight appear to have an important correlation with the spring maximum of plankton, but can hardly be held accountable for the October and November increase of plankton.

Bacteria showed two major peaks of abundance throughout the year. The maximum occurred in September and the minimum in December. The September maximal and also the minimal pulses of bacteria between May and October appear to be in direct correlation with the plankton pulses, and generally follow the plankton pulses with a lag (graphs 8 and 7). It does not appear likely that the September peak could be attributable to the fall turnover of the lake, since it precedes the turnover. We may assume then, perhaps, as does Henrici (10), that the product of phytoplankton decay "is an important factor in determining the number of bacteria."

SUMMARY AND CONCLUSIONS

1. This study revealed a marked periodicity both of the total phytoplankton, and of the classes, genera, and species of the algæ.

2. The classes in order of numerical abundance for the year were, Bacillariophyceæ, Chrysophyceæ, Myxophyceæ, Chlorophyceæ, and Dinophyceæ.

3. The total phytoplankton showed considerable weekly variation, and it exhibited a spring and autumn maximum, which occurred respectively in June and October. Both of the maxima were attributable to diatoms, and especially to Synedra.

4. The maximal monthly average production of total phytoplankton occurred in June. The minimal monthly average production occurred in March.

5. The maxima of total phytoplankton were augmented by pulses of each of the dominant diatoms, which were Asterionella, Fragilaria,

Melosira, Synedra, and Tabellaria. Codominants were Cyclotella, Navicula, and Rhizosolenia.

6. Each of the phytoplanktonts displayed its own pulses throughout the year. Likewise, species of the same genus were usually much unlike in numbers at the same weekly intervals throughout the year.

7. The peaks of abundance of the dominant genera of diatoms occurred in the following order: Melosira in May, Asterionella and Synedra in June, and Fragilaria and Tabellaria in July.

8. The curve for diatoms conforms almost exactly with that of total phytoplankton.

9. Among the dominant genera and species of diatoms from June until December, there was at least one distinct pulse and sometimes more than one in each month.

10. The order Pennales led the Centrales in number and species at all times.

11. The class Chrysophyceæ was second in abundance to the Bacillariophyceæ, and the order Chrysomonodales, which contains Dinobryon, was most abundant.

12. The Myxophyceæ appeared to have two periods of abundance, the higher in September and the lower in June. The order Hormogonales, represented chiefly by Lyngbya, was the outstanding group.

13. Chlorophyceæ, represented mainly by Ankistrodesmus of the order Chlorococcales, and Dinophyceæ, represented by Ceratium and Peridinium, were never abundant in the samples.

14. Hydrogen-ion concentration appears to have little, if any, appreciable effect on seasonal periodicity of the phytoplankton. Hydrogen-ion concentration varies from 7.4 to 8.0 throughout the year.

15. Although temperature is important in optimum growth of most phytoplanktonts, it does not seem to be of prime importance as a controlling factor in diatom periodicity.

16. Turbidity, caused by lake turnovers in the spring and fall, and by storm and wind between periods of turnovers, seems to exert a very important influence on seasonal growth and pulses.

17. Hours of sunlight appear to have an important correlation with the spring maximum of plankton, but hardly can be held accountable for the October and November increase of plankton.

18. The September maximum and also the minimal pulses of bacteria between May and October appear to be in direct correlation with the plankton pulses and generally follow these plankton pulses with a lag.

A SYSTEMATIC LIST OF THE PHYTOPLANKTON

Bacillariophyceæ
- Order Centrales
  - Cyclotella glomerata Bachmann
  - Cyclotella melosiroides (Kirchner) Lemmermann
  - Cyclotella
  - Melosira
  - Rhizosolenia
  - Stephanodiscus
- Order Pennales
  - Amphiprora ornata Bailey
  - Amphora ovalis Kütz.
  - Asterionella
  - Cocconeis
  - Cymatopleura solea (Breb.) W. Smith
  - Cymatopleura elliptica (Breb.) W. Smith
  - Cymbella
  - Diatoma
  - Fragilaria crotonensis Kitton
  - Fragilaria
  - Gomphonema
  - Gyrosigma
  - Navicula
  - Nitzschia
  - Pinnularia
  - Surirella ovata Kütz.
  - Synedra radians Kütz.
  - Synedra ulna (Nitzsch.) Ehr.
  - Synedra
  - Tabellaria flocculosa (Roth) Kütz.
  - Tabellaria fenestrata Kütz.

Chrysophyceæ
- Order Chrysomonodales
  - Chrysosphærella longispina Lauterb.
  - Chrysosphaerella longispina Lauterb.
  - Dinobryon
  - Mallomonas

Myxophyceæ
- Order Chroöcoccales
  - Aphanocapsa
  - Chroöcoccus
  - Cœlosphærium
  - Gomphosphæria lacustris Chod.
  - Microcystis æruginosa Kütz.
  - Microcystis
- Order Hormogonales
  - Anabæna
  - Lyngbya
  - Oscillatoria

Chlorophyceæ
- Order Chlorococcales
  - Ankistrodesmus
  - *Cœlastrum cambricum Arch.
  - Dictyosphærium pulchellum Wood
  - Golenkinia
  - Oöcystis
  - Pediastrum
  - Scenedesmus
- Order Desmidiales
  - Closterium
- Order Tetrasporales
  - *Glœocystis planctonica (W. & G. S. West) Lemmermann
  - Sphærocystis Schroeteri Chod.
- Order Zygnematales
  - *Spondylosium pygmaeum (Cooke) W. West

Dinophyceæ
- Ceratium hirundinella (O. F. M.) Schrank
- Peridinium

*New species for Lake Michigan.

ACKNOWLEDGMENTS

The writer expresses his sincere gratitude to Dr. L. H. Tiffany, under whose direction this research was carried out, for aid in identification and for generous critical suggestions in preparing the manuscript. The writer is indebted to E. S. Ford for aid in the early part of the investigation and for making the weekly plankton collections and filtrations from June 4 to September 15, 1937.

Thanks are expressed to Dr. Glenn C. Couch, of the Oklahoma University Department of Botany, for aid in identification of diatoms and for helpful suggestions; to Horace Frye, assistant superintendent of the Evanston Water Department, for preparing the turbidity standards, for aid in securing literature, and for permitting use of scientific data which were recorded at the Filtration Plant; and to Dr. R. C. Friesner for the use of laboratory and equipment in the Butler University Department of Botany for identification of phytoplankton during the summer months of 1937.

LITERATURE CITED

1. Ahlstrom, Elbert H. The deep-water plankton of Lake Michigan, exclusive of the Crustaceæ. Trans. Amer. Mic. Soc. 55:286-299. 1936.
2. Allen, W. E. A quantitative and statistical study of the plankton of the San Joaquin river and its tributaries in and near Stockton, California, in 1913. Univ. California Pub. Zoöl. 22:1-292. 1920.
3. Baylis, John R., and H. M. Gerstein. Micro-organisms in the lake water at Chicago. Municipal News and Water Works. 291-296. July 1929.
4. Briggs, S. A. The Diatomaceæ of Lake Michigan. The Lens 1:41-44. 1872.
5. Burkholder, Paul R. Microplankton studies of Lake Erie. Bull. Buffalo Soc. Nat. Sci. 14:73-93. 1929.
6. Coffing, Charlene. A quantitative study of the phytoplankton of the White river canal, Indianapolis, Indiana. Butler Univ. Bot. Stud. 4:13-31. 1937.
7. Eddy, Samuel. The plankton of Lake Michigan. Illinois State Nat. Hist. Surv. Bull. 17:203-232. 1927.
8. —— A study of fresh-water plankton communities. Illinois Biol. Mono. 12:1-93. 1934.
9. Gottschall, R. Y., and O. E. Jennings. Limnological studies at Erie, Pennsylvania. Trans. Amer. Mic. Soc. 52:181-191. 1933.
10. Henrici, Arthur T. Studies of freshwater bacteria. IV. Seasonal fluctuations of lake bacteria in relation to plankton production. Jour. Bact. 35:129-139. 1938.
11. Jennings, H. S. Report on the Rotatoria. Appendix 3. Biological examination of Lake Michigan. Bull. Michigan Fish Comm. 6:85-93. 1896.
12. Kofoid, C. A. The plankton of the Illinois river, 1894-99, with introductory notes upon the hypography of the Illinois river and its basin. Part I. Quantitative investigations and general results. Bull. Illinois State Lab. Nat. Hist. 6:95-629. 1903.
13. —— The plankton of the Illinois river. 1894-1899. Part II. Constituent organisms and their seasonal distribution. Bull. Illinois State Lab. Nat. Hist. 8:1-360. 1908.
14. —— Report on the Protozoa. Appendix II. Bull. Michigan Fish Comm. 6:76-87. 1896.
15. Pearsall, W. H. A theory of diatom periodicity. Jour. Ecol. 11:165-183. 1923.
16. —— Phytoplankton in the English lakes. II. The composition of the phytoplankton in relation to dissolved substances. Jour. Ecol. 20:241-262. 1932.
17. Roach, Lee S. An ecological study of the plankton of the Hocking river. Ohio Biol. Surv. 5:253-279. 1932.
18. Standard methods of water analysis. Eighth Ed. 1936.
19. Tiffany, L. H. Algæ, the grass of many waters. Chas. C. Thomas Co. (In press.)
20. Thomas, B. W., and H. H. Chase. Diatomaceæ of Lake Michigan during the last 16 years from the water supply of the city of Chicago. Notarisia 2:328-330. 1887.
21. Thompson, H. D. Report on the plants. Appendix I. Bull. Michigan Fish Comm. 6:72-75. 1896.
22. Todd, James Campbell, and Arthur Hawley Sanford. Clinical diagnosis by laboratory methods. W. B. Saunders Co., Philadelphia and London. 1934.

23. Ward, H. B. A biological examination of Lake Michigan in the Traverse bay region. Bull. Michigan Fish Comm. 6:1-71. 1896.
24. Welch, Paul S. Limnology. McGraw Hill Book Co., Inc., New York and London. 1935.
25. West, W., and G. S. West. Periodicity of the phytoplankton. Jour. Linn. Soc. Bot. 40:393-432. Review in Jour. Ecol. 1:204-205. 1913.
26. Whipple, G. C. Microscopy of drinking water. John Wiley & Sons, New York; Chapman & Hall, London. 1927.

A COMPARISON OF MARKET MILK FROM TEN INDIANAPOLIS COMPANIES BY USE OF THE DIRECT MICROSCOPIC METHOD OF ANALYSIS[1]

By Ina Stanley

The part played by milk in the health and daily life of individuals is of such importance that production of clean, safe milk presents a problem which should by no means be neglected. Steps taken to safeguard consumers have included medical examination of cows, examination for dirt through the sediment test, examination for bacteria by means of the agar plate method, and, more recently, also through direct microscopic examination.

The United States Government has undertaken to regulate practices connected with milk production on the farm, as well as with the handling of milk in pasteurizing and bottling plants (1). These regulations have helped matters greatly in towns where they are enforced. A preference for milk produced under these regulations was shown during the 1937 Ohio River flood, when Louisville sent to Chicago for its milk supply instead of taking the much closer Indianapolis milk, where no federal standards are enforced.

The fact that Indianapolis milk is not produced under Government regulation might lead to great differences in the quality of milk put out by various companies. With this thought in mind, the following experiment was undertaken to compare milk from ten of the Indianapolis companies as it reached the consumer, the work being carried on in the Botany Department laboratories of Butler University.

METHODS

Both the agar plate and direct microscopic methods were used. In the latter, the object was to examine samples for morphological types of bacteria as well as numbers.

One quart bottle of milk, delivered by each of the ten companies to homes, was obtained and taken to the laboratory for examination. This was done on two different days, one in July and one in September of 1937. The ten dairies are not listed by name in this report, but each

[1]This paper is a portion of a thesis in partial fulfillment of the requirements for graduation *magna cum laude* in Butler University.

is designated by a letter of the alphabet, since the limited number of tests made, with obviously an incomplete record, makes this desirable.

Both plate and direct microscopic methods were used. The agar used for the plate method was made according to the formula recommended in Standard Methods (2). Sterile plates, pipettes, water blanks, and agar were used. Dilutions of 1:100, 1:1000, and 1:10,000 were plated and incubated at 37° C. for 48 hours.

For the direct method, .01 cc of whole milk was taken in a calibrated pipette, deposited on a clean slide, and spread over an area of one square centimeter with a sterile needle. The preparation was dried, placed in xylol for five minutes to remove the fat, dried again and dipped in 95 percent alcohol for five minutes to fix the material to the slide. After drying again, the slide was dipped in a saturated aqueous solution of methylene blue for 2-5 seconds. Where necessary, it was destained in 95 percent alcohol (3).

A binocular microscope with 6X oculars and 1.8 immersion oil objective was used for counting the bacteria. This gave a field of 1/3000 square centimeters with a diameter of 205 microns, making it possible to examine 1/300,000 of a cubic centimeter of milk in each field. Thirty fields of each sample were observed for number of body cells, total number of bacteria, number of clumps of bacteria, types of bacteria (whether staphylococcus, streptococcus, diplococcus, or rod forms), and fungi. All chains of streptococci consisting of eight cells or over were considered long-chain forms, while those under eight cells were recorded as short streptococci.

RESULTS

Readings for the plate count were made from the 1/1000 dilutions, since colonies on the other dilutions were too few or too many to be very accurate. These counts ranged from 4000 to 120,000 in the July count and from 1000 to 198,000 in the September count. Company J was high both times, with companies B and F lowest on the first count and company A lowest in September.

Results of the plate count and total microscopic count for July and September are shown in table I. It will be seen that companies H and J keep about the same position in respect to the other companies in all four counts, H having a low count, while J had the highest in all cases. In the plate count, six of the companies showed an increase in number of bacteria in September over that found in July, while four showed a

TABLE I

Total Bacterial Counts

Company	Direct Count		Plate Count	
	July	September	July	September
A	10,360,000	2,840,000†	41,000	1,000†
B	11,270,000	4,170,000	4,000†	15,000
C	17,410,000	10,380,000	14,000	54,000
D	8,100,000†	6,120,000	16,000	2,000
E	17,010,000	11,250,000	17,000	190,000
F	12,450,000	6,100,000	4,000†	5,000
G	15,950,000	6,390,000	5,000	74,000
H	9,800,000	4,080,000	5,000	2,000
K	10,550,000	7,800,000	42,000	5,000
J	31,910,000*	20,930,000*	120,000*	198,000*

*Highest Count. †Lowest Count.

decrease. Four of those companies showing an increase showed in general a high count, while the other two are medium.

In examining the milk microscopically, it was found that all counts dropped in September. In ranking the companies according to plate and microscopic count, only slight differences were found. Those showing high plate counts also showed high microscopic counts with the exception of F, which showed a low plate and high microscopic count when compared with the other companies.

In examining for the various types of bacteria, it was found that J had a larger number of all forms in all cases except the July count of short streptococci. E, K, and J all showed a considerable amount of mold mycelium in both July and September counts. C and J showed over 3,000,000 body cells each for July, J showing over 6,000,000.

A, C, and J showed more of the staphylococci than other forms in both samples, and the July sample of A was also high in long streptococci. Staphylococci were also found to form the largest group in September J and July D. In all other cases, diplococci formed the largest group. Streptococci were not numerous in comparison to the other types except in J, both samples, and in E and H for July.

Table II shows the numbers of each kind of bacteria per company.

DISCUSSION

The direct microscopic method is rarely used by dairymen on pasteurized milk, but in many cases forms part of the test of raw milk

TABLE II

BACTERIA COUNT ACCORDING TO TYPE—SUM OF 30 FIELDS

	Staphylococci		Diplococci		Long Strep.		Short Strep.		Body Cells		Fungus	
COMPANY	July	Sept.	July	Sept.	July	Sept.	July	Sept.	July	Sept.	July	Sept.
A	504	152	406	112†	16	.....†	105	12†	202	138	.....†	.....†
B	456	141†	564	240	10†	8	65	25	170	152	2	6
C	1009	588	550	290	111	42	60	116	339	166	2	1
D	400	208	338	332	32	16	34	43	187	176*	1	3
E	638	374	848	616	42	32	165*	69	247	156	18*	16
F	881	396	302†	184	10†	8	40†	17	254	154	.....†	4
G	596	199	822	486	64	8	92	27	173	134	2	5
H	168†	151	306	174	200	32	232	46	258	89†	.....†	.....†
K	379	336	493	330	82	12	76	97	126†	100	7	15
J	1510*	998*	1125*	624*	420*	277*	124	192*	654*	176*	9	24*

†Lowest Count. *Highest Count.

These numbers can be multiplied by 10,000 to give the number per cc.

as it enters the dairy. Although the quality of milk at the time it reaches the dairy is of great importance, the quality of milk when it is delivered to the consumer is more important to the latter. A direct microscopic count made of the milk as it is delivered can give a fairly accurate history of the milk from the time it was drawn until it is delivered. This history is provided by the bacteria themselves, since each type of organism is an indicator of conditions surrounding the milk production and distribution.

The diplococci are paired, spherical bacteria, and are the normal flora of milk, being known as the lactic acid bacteria. Under proper conditions for growth, these are the forms which cause milk to sour. This growth is accelerated by slow cooling. The streptococcic form occurring in milk as double pairs or chains of spherical bacteria are also indicators of slow cooling. They do not regularly form long chains. Long chains of spherical streptococci associated with an excessive number of body cells, *i. e.*, over 3,000,000 per cc, indicates mastitis. This is an abnormal condition of the udder, due to infection through the teat canal of individual quarters of the udder.

Staphylococci are indicators of unclean utensils. They originate with the producer in improperly cleaned milking machines and in crevices and open seams of cans. They may also come from the dairy through improperly cleaned pipes and pipe connections as well as unclean bottles.

The common milk mold is *Oospora lactis*. It is seldom observed in fresh milk (4). This mold is one commonly found around farms, and

in milk which has come into contact with some surface containing a scum of sour milk solids. It is found associated with lactic-acid bacteria in filthy cans, milking machine tubes, and connections. Other types of mold mycelium gain entrance from feed in cases where the cows are fed while being milked (4).

Interpreting the results of this experiment in the light of what has been said above brings out certain interesting points. The staphylococcic and diplococcic forms are most numerous in all cases except the July count for H, in which case the diplococci form the greatest group and staphylococci the smallest. Normally the diplococci should form the largest group, but A, C, and F had staphylococci predominating in both counts. This was also true of the July count of D and September count of J, indicating utensil contamination. In all other cases, diplococci formed the largest group.

Company H had a consistently low count in diplococci and staphylococci, but a higher count in streptococci than some other companies, indicating poor cooling, which may take place either at the time the milk is drawn or after pasteurization. Poor cooling was apparently a great factor in all cases, since the number of diplococci and streptococci ran high in all samples. It was to be expected that the September count would be lower than July, due to cooler weather. This was found true in all cases, but the September count of diplococci was higher in some cases than that of July for other companies. This also indicates lack of care on the part of the former producers in cooling.

The fungus count was negligible for all companies except E, K, and J. Producers here either fed their animals during milking or were not careful in guarding the supply from other external contamination.

Mastitis was indicated by only one sample. This was in J for July. Milk from C had a large number of body cells in July, but not an unusually large amount of streptococci. It is not probable that a composite sample from many cows would show much mastitis, therefore this condition must have been present in almost all of the herd in order to show here.

Company A showed no fungi, few streptococci, and more staphylococci than diplococci. The total count was rather low. This indicates care in cooling, but that more care should be taken in keeping utensils clean. B showed large numbers of staphylococci and diplococci, with some fungi. Care should probably be taken here in feeding during milking, quicker cooling, and keeping utensils clean. C showed large numbers of staphylococci and diplococci as well as streptococci. This indicates

slow cooling and dirty utensils. Large numbers of staphylococci and diplococci were shown in D, indicating unclean utensils and poor cooling. E showed many more diplococci than staphylococci, and several pieces of fungi, indicating slow cooling and lack of care in cleaning utensils or feeding during milking. F showed its total number of bacteria composed mostly of staphylococci, indicating unclean utensils. G showed large numbers of both staphylococci and diplococci, indicating slow cooling and unclean equipment. H showed low counts generally and, in comparison with the others, showed up best, but improvement could be made, apparently, in cooling and cleaning utensils. This is true also of K, which showed a fairly low count. J showed the highest count, and all things which have been mentioned as going together to make up milk production could evidently be much improved here, as the condition generally seemed very poor.

The plate and direct microscopic counts both give only estimates of the total number of bacteria present, but the latter is much more accurate, as was shown by the consistency in the two direct counts and lack of consistency in the plate counts. This is true because the plate method counts only colonies, each one of which may have started from any number of individual cells. The microscopic method is also easy and quick. Only a few minutes are required in the latter method to determine with what sort of milk one is dealing. If the sample is not of a high quality, the cause can be quickly determined and remedied. In order to learn anything from a plate count, two days must elapse for incubation, and by that time any undesirable milk which might be discovered will have been distributed to the consumer.

Good quality milk should be clean and not cleaned. The microscopic count shows particles of dirt which are not removed in filtering, and which would not be noticeable by any other method. One difficulty with the microscopic test is that it shows organisms which are dead after pasteurization as well as those which are living. According to Lazarus (4), the dead can be distinguished by the fact that they take a much lighter stain than the living; however, no such distinction was made in this paper.

The number of bacteria in milk is no index as to its safeness, but it does give an idea of what its keeping qualities are, and in what sort of surroundings it was produced. Surroundings giving rise to large numbers of bacteria would probably be the place in which contamination of a more serious nature would arise.

Pasteurization has been known to cut the count from 10,000 to 500

bacteria per cc (plate count), but it is possible for the process to increase the count. Certain forms of bacteria, mostly spore-formers, are able to endure the heat, and even to multiply in its presence. There are also many steps in the process where bacteria can be added to the milk, the pipes and connections being very important in this respect. Improvements are being constantly made, however, which go a long way toward solving the problem.

TABLE III—SUMMARY

AVERAGE OF JULY AND SEPTEMBER FIGURES (IN 10,000's PER CC)

Rating	Milk Company	Direct Count	Plate Count	Diplococci	Short Streps	Long Streps	Body Cells	Staphylococcus	Mold
Best	H	694	.4	240	139	116	174	160	....
Fair	A	660	2.1	259	59	8	170	328	....
	D	711	.9	335	39	24	182	304	2
	F	928	.5	243	29	9	204	639	2
Mediocre	B	772	1.0	402	45	9	161	299	4
	K	918	2.4	414	87	47	113	358	11
Poor	G	1117	4.0	654	60	36	154	398	4
	C	1390	3.4	420	85	77	253	799	2
	E	1413	10.4	732	117	37	202	506	17
Worst	J	2642	15.9	875	158	349	415	1254	17

SUMMARY

In comparing the companies, H appeared to deserve first ranking because of its consistently good quality. Although other companies surpassed it in some features, they fell so low in others that they could not be given a first-place rating.

There was no doubt as to which ranked lowest, since J kept that position consistently. This milk evidently came from a dairy which was neglectful and unsanitary in several respects.

Taking the total counts of the other companies for both plate and microscopic examinations for July and September, the other companies could be ranked as follows: In the second class were placed three companies, A, D, and F. These all showed a fairly high quality milk in comparison to the others. In the mediocre group were placed B and K, while in the poor group were placed G, C, and E. G had the lowest count and E the highest of that group.

In general, all companies could lower their count considerably by

quicker and better cooling methods, and by taking greater care in cleaning all apparatus, including that used before the milk reaches the dairy.

LITERATURE CITED

1. United States Treasury Department. Milk ordinance and code: 1936. Public Health Bulletin 220. 1937.
2. American Public Health Association. Standard methods of milk analysis. American Public Health Association. 1934.
3. Robertson, A. H. The relation between bacterial counts from milk as obtained by microscopic and plate methods. New York Agric. Exp. Sta. Tech. Bull. 86. 1921.
4. Lazarus, N. E. Quality control of market milk. Olsen Publishing Co. 1935.
5. Ernst, W., J. R. Mohler, and A. Eichhorn. Textbook of milk hygiene. Alexander Eger. 1915.